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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/669,976	ENGEL ET AL.	
	Examiner	Art Unit	
	STEPHANIE K. MUMMERT	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 April 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2,4-16 and 25-28* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,4-16 and 25-28* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on April 6, 2009 has been entered.

Applicant's amendment filed on April 6, 2009 is acknowledged and has been entered. Claims 1-2, 4-6, 25 have been amended. Claim 3 and 23-24 have been canceled. Claims 26-28 have been added. Claims 1-2, 4-16 and 25-28 are pending. Claims 17-22 are withdrawn from consideration as being drawn to a non-elected invention.

All of the amendments and arguments have been thoroughly reviewed and considered but are not found persuasive for the reasons discussed below. Any rejection not reiterated in this action has been withdrawn as being obviated by the amendment of the claims. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1-2, 4-16 and 25-28 are discussed in this Office action.

This action is made NON-FINAL as necessitated by New Grounds of Rejection

Previous Grounds of Rejection

The objection to the specification as improperly incorporating subject matter is withdrawn in view of the amendment to the specification.

The statements of rejection have been updated to correct a typographical error in the patent number associated with Backus, consistent with the number provided on the references cited.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

1. Claims 1-2, 4-11, 16 and 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and further in view of Birch et al. (US Patent 5,773,258; June 1998). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

With regard to claim 1, Backus teaches a method for the coamplification of two or more target nucleic acids having different sequence compositions, said method comprising at least 15 primary amplification cycles (col. 2, line 67 to col. 3, line 3), each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture comprising two or more target nucleic acids and a hot start DNA polymerase, or their primer extension products, at a first temperature, T1, for denaturation of the strands of the target nucleic acids or their primer extension products (col. 3, lines 4-8; col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T2 (col. 3, lines 9-12), and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T3, provided that when priming and primer extension product formation are carried out in the same step, T2 and T3 are the same (col. 3, lines 13-18),

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting two or more of the primer extension products having different sequence composition as an indication of coamplification of the target nucleic acids (col. 3, lines 46-48); an improvement comprising using the DNA polymerase included in the reaction mixture of step (A), a modified thermostable hot start DNA polymerase enzyme that becomes active only after incubation at temperatures above 50oC (col. 3, lines 4-8; col. 7, lines 11-19, where the inclusion of a polymerase and the inclusion of an antibody specific to the DNA polymerase which inhibits enzymatic activity below about 50°C and which is inactivated at higher temperatures), and using in at least one of the primary amplification cycles, 1 to 20 weight

% of a nonionic polymeric volume exclusion agent (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 2, Backus teaches an embodiment of claim 1, wherein the amount of polymeric volume exclusion agent in said reaction mixture is 1 to 15 weight % (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 4, Backus teaches an embodiment of claim 1, wherein the amount of polymeric volume exclusion agent in said reaction mixture is 1 to 8 weight % (col. 3, lines 19-21, where the disclosed percentage of 4 weight % falls within the range(s) claimed).

With regard to claim 5, Backus teaches a method according to one of claims 1, 2 or 4, characterized in that the volume exclusion agent is selected from the group consisting of a polyether, a reaction product of a sugar with epichlorohydrin, a polysaccharide, and a polyacrylate (col. 7, lines 36-41).

With regard to claim 6, Backus teaches a method according to claim 5, characterized in that the volume exclusion agent is selected from the group of polyethers of the general formula;

$$H\text{-}(\text{-}O\text{-}R\text{-})_n\text{-}H$$

wherein R is an alkylene bridge of 1 to 6 carbon atoms - branched or unbranched - and n is an integer of 15 to 1000 (col. 3, lines 42-48).

With regard to claim 7, Backus teaches an embodiment of claim 6, characterized in that R may represent 1,2-ethylene, 1,3-propylene, 1,2-propylene, 2-hydroxy-1,3-propylene, 3-hydroxy-1,2-propylene, 1,4-butylene, 1,3-butylene, or 1,2-hexylene (col. 7, lines 48-52).

With regard to claim 8, Backus teaches an embodiment of claim 6, characterized in that

the polyether is poly(ethylene glycol) (col. 7, lines 53-56, where it is noted that a preferred R group is polyethylene glycol).

With regard to claim 9, Backus teaches an embodiment of claim 8, characterized in that the polytethylene glycol) has a molecular weight in the range of 1000 daltons to 2,000,000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 10, Backus teaches an embodiment of claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 3000 daltons to 500,000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 11, Backus teaches an embodiment of claim 8, characterized in that the poly(ethylene glycol) has a molecular weight of about 8000 daltons (col. 7, lines 59-67, where it is noted that the preferred range is between about 1000 to about 20,000, wherein the term about represents +/-10% and wherein the preferred range of molecular weights falls within the range as claimed, presuming that it is intended for the claimed ranges to be measured in

Daltons; col. 15, lines 19-21, where PEG-8000, a polyethylene glycol with a molecular weight of 8000 daltons was used in the examples).

With regard to claim 16, Backus teaches an embodiment of claim 5, characterized in that the polyacrylate is selected from the group consisting of poly(hydroxyethyl acrylate) or poly(2,3-dihydroxypropyl acrylate) (col. 8, lines 12-15).

Regarding claims 1, 2 and 4, while Backus teaches a reversibly modified thermostable DNA polymerase, Backus does not teach a modification that comprises a chemical modification as established in the specification. Birch teaches the reversible modification of DNA polymerase by an inhibiting agent (Abstract).

With regard to claims 1, 2 and 4, Birch teaches a chemically-modified thermostable hot start DNA polymerase enzyme that becomes active only after incubation at temperatures above 50°C (Abstract; col. 4, lines 49-58, where the reversibly inactivated enzyme is a thermostable DNA polymerase; col. 3, lines 1-19, where a DNA polymerase is reversibly inactivated using treatment with a modifier reagent and becomes active at a temperature of about 50 °C, col. 3, lines 44-51).

Furthermore, regarding claims 1, 2 and 4, neither Backus nor Birch explicitly teach that the two or more target nucleic acids are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold. Regarding claims 2 and 4, Backus does not teach that the reaction mixture comprises a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization.

Bustin teaches an overview of the quantitation of mRNA using a variety of methods, including quantitative real-time RT-PCR, a method which incorporates a variety of means of detection, including hybridization probes (Abstract).

With regard to claims 1-2 and 4, Bustin teaches the coamplification of nucleic acids which are present at comparable copy numbers, wherein the maximum difference between the lowest and highest copy number is 10-fold (p. 182, col. 1, 2nd paragraph, where the normalization of quantification of a target nucleic acid is accomplished through co-amplification of an internal control target sequence, referred to as an endogenous control. It is also noted that the endogenous control should be expressed at roughly the same level as the RNA under study; see also p. 185, 'multiplex RT-PCR' heading, where multiple primer sets are used to amplify multiple specific targets simultaneously).

With regard to claim 1, Bustin discloses a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization (Figure 3 and p. 174, where molecular beacon probes were described; Figure 4A-C right side and p. 177, where 'hybdrization probes' were described; Figure 5, p. 177, where TaqMan hydrolysis probes were described).

With regard to claim 28, Bustin teaches an embodiment of claim 1, characterized in that the sequence specific labeled probe is fluorescently labeled (Figure 3 and p. 174, where molecular beacon probes were described and are fluorescently labeled; Figure 4A-C right side and p. 177, where 'hybdrization probes' were described; Figure 5, p. 177, where TaqMan hydrolysis probes were described).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Backus to incorporate the fluorescent hybridization probes and coamplification of a target sequence of comparable copy number of Bustin, to arrive at the claimed invention with a reasonable expectation for success. As taught by Bustin, "The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study" (p. 182, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number.

Furthermore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an chemical as taught by Birch to arrive at the claimed invention with a reasonable expectation for success. The reversible modification taught by Backus includes "an antibody specific to the DNA polymerase, which antibody inhibits enzymatic activity at a temperature below about 50°C, but which antibody is deactivated at higher temperatures" (col. 7, lines 11-20). In a related technique, Birch teaches methods that

"use a reversibly inactivated thermostable enzyme which can be reactivated by incubation in the amplification reaction mixture at an elevated temperature" (col. 2, lines 62-65). Birch also teaches a preferred embodiment wherein "the amplification reaction is a polymerase chain reaction (PCR) and a reversibly-inactivated thermostable DNA polymerase is used. The reaction mixture is incubated prior to carrying out the amplification reaction at a temperature which is higher than the annealing temperature of the amplification reaction. Thus, the DNA polymerase is inactivated until the temperature is above the temperature which insures specificity of the amplification reaction, thereby reducing non-specific amplification" (col. 4, lines 49-58). Both Backus and Birch teach modification that is reversible with an increase in temperature. Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with a chemical as taught by Birch to arrive at the claimed invention with a reasonable expectation for success.

2. Claims 12-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applies to claims 1-2, 4-11, 16 and 28 and further in view of Reed et al. (US Patent 5,459,038; October 1995) and Demke et al. (Biotechniques, 1992, vol. 12, no. 3, p. 333-334). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

Regarding claims 12-15, while Backus teaches amplification in the presence of Dextran sulfate as inhibiting to amplification, Demke provides an explanation that while dextran sulfate is inhibitory to PCR amplification, Dextran does not inhibit amplification via PCR.

Demke does not provide explicit teaching that dextran provides an improvement to PCR amplification without the inclusion of a volume exclusion agent. Reed teaches amplification of samples in the presence of Dextran (col. 19, lines 9-26, where the inclusion of Dextran results in more efficient amplification).

With regard to claim 12, Reed teaches an embodiment of claim 5, characterized in that the volume exclusion reagent is a dextran (col. 19, lines 9-26, where the inclusion of Dextran results in more efficient amplification).

With regard to claim 13, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 1000 to 2,000,000 (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000).

With regard to claim 14, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 3000 to 500,000 (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000).

With regard to claim 15, Reed teaches an embodiment of claim 12, characterized in that the dextran has a molecular weight in the range of 40,000 daltons to 60,000 daltons (col. 16, lines 10-20, where the dextran is T500, which comprises a MW of 500,000, see obviousness rejection below).

While Reed teaches a Dextran of molecular weight 500,000, Reed also teaches that dextran generally provides an improvement over PCR amplification reactions that are not

conducted in the presence of dextran. Thus, an ordinary practitioner would have recognized that the results optimizable variables of time, concentration and product amount could be adjusted to maximize the desired results. As noted in *In re Aller*, 105 USPQ 233 at 235,

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the results were other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Backus to incorporate the fluorescent hybridization probes and coamplification of a target sequence of comparable copy number the of Bustin, to arrive at the claimed invention with a reasonable expectation for success. As taught by Bustin, "The accepted method for minimizing these errors and correcting for sample-to-sample variation is to amplify, simultaneously with the target, a cellular RNA that serves as an internal reference against which other RNA values can be normalized. The ideal internal standard should be expressed at a constant level among different tissues of an organism, at all stages of development, and should be unaffected by the experimental treatment. In addition, an exogenous control should also be expressed at roughly the same level as the RNA under study" (p. 182, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with a reasonable expectation for

success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number.

Furthermore, it would have been prima facie obvious in view of the teachings of Demke and Reed to include dextran into the method of amplification taught by Backus in view of Bustin. First, it is noted that Backus teaches amplification in the presence of PEG and dextran sulfate. While Backus teaches that dextran sulfate is inhibitory to amplification, Demke teaches "the inhibitory nature of some polysaccharides with free acidic groups is further demonstrated by contrasting dextran and dextran sulfate. Dextran (neutral) has no interfering effects at 500:1 ratio, wheras dextran sulfate was very inhibitory (Table 1). Therefore, considering the teachings of Demke, it would have been prima facie obvious to substitute the dextran sulfate taught by Backus for the equivalent dextran as taught by Demke. Furthermore, as taught by Reed, "the inclusion of polysaccharide dextran (or similar) results in three unique advantages: firstly, its inclusion results in more efficient amplification leading to markedly higher sensitivity and specificity" (col. 19, lines 9-26). Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have extended the method taught by Backus to include dextran as taught by Reed and Demke to achieve efficient amplification with higher sensitivity and specificity with a reasonable expectation for success.

3. Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28 above, and further in view of Ivanov et al. (US Patent 6,183,998; February

2001). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

Backus in view of Bustin and Birch renders obvious claims 1-2, 4-11, 16 and 28 as recited in the 103 rejection stated above. While Backus in view of Bustin and Birch teaches a chemically modified DNA polymerase, neither Backus, Bustin or Birch teaches that the modification is due to a reaction with an aldehyde. Ivanov teaches reversible modification of DNA polymerases through reaction with an aldehyde (Abstract).

With regard to claim 25, Ivanov teaches an embodiment of claim 1, 2 or 4, wherein said chemically modified DNA polymerase is modified by reaction with an aldehyde (Abstract).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an aldehyde as taught by Ivanov to arrive at the claimed invention with a reasonable expectation for success. The reversible modification taught by Backus includes "an antibody specific to the DNA polymerase, which antibody inhibits enzymatic activity at a temperature below about 50°C, but which antibody is deactivated at higher temperatures" (col. 7, lines 11-20). In a related technique, Ivanov teaches "for reversible inactivation of thermostable enzymes using a chemical modification under essentially aqueous conditions. In particular, the thermostable enzymes of the present invention are reversibly modified in the presence of an aldehyde". Furthermore, Ivanov teaches "enzymatic activity of the present chemically modified enzymes is increased at least two-fold within thirty minutes when incubated at a more elevated temperature, i.e. above 50 °C, preferably at a temperature of 75 °C to 100 °C" (col. 3, lines 1-14). Both Backus and Ivanov teach modification that is

reversible with an increase in temperature. Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have extended the teachings of Bustin and Backus to include the chemical modification through reaction with an aldehyde as taught by Ivanov to arrive at the claimed invention with a reasonable expectation for success.

New Grounds of Rejection as necessitated by Amendment

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. Claims 26-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Backus et al. (US Patent 5,705,366; January 1998) in view of Bustin, SA (Journal of Molecular Endocrinology, 2000, vol. 25, p. 169-193) and Birch et al. (US Patent 5,773,258; June 1998) as applied to claims 1-2, 4-11, 16 and 28 above and further in view of Mansfield et al. (Molecular Cellular Probes, 1995, vol. 9, p. 145-156). Backus discloses a method of amplification of multiple target nucleic acids in the presence of a nonionic, polymeric volume exclusion agent (Abstract).

5. Backus in view of Bustin and Birch render obvious all of the limitations of claims 1-2, 4-11, 16 and 28. However, neither Backus, Bustin or Birch teach that one of the primers is fluorescently labeled or that one of the primers is labeled with a specific binding moiety. Mansfield teaches a variety of primer labeling techniques (Abstract).

With regard to claim 26, Mansfield teaches an embodiment of claim 1, characterized in that one of the primers of each primer set is fluorescently labeled (p. 145, col 2; p. 146, col. 1, where PCR incorporating a 5' end tagged primer sequence labeled with a fluorescent nucleotide is described).

With regard to claim 27, Mansfield teaches an embodiment of claim 1, characterized in that one of the primers of each primer set is labeled with a specific binding moiety (p. 145, col 2, where a small molecule like fluorescein, biotin or digoxigenin can be incorporated into the detection reagent; Figure 1, where the biotin label is attached to a probe, but the attachment of a probe or a primer is equivalent).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the teachings of Backus, Bustin and Birch to include the labeled primers of Mansfield to arrive at the claimed invention with a reasonable expectation for success. As taught by Mansfield, "fluorescent, chemiluminescent, bioluminescent and colorimetric approaches have been used as alternatives to radioactive detection methods. Fluorogenic or chemiluminescent or chemiluminescent substrates allow subattomole level detection of DNA labels in solid-phase membrane-based hybridization assays with much greater sensitivity than using colorimetric methods". Mansfield also emphasizes that "fluorescence detection is by far the most versatile" (p. 154, col. 1). Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to have adjusted the teachings of Backus, Bustin and Birch to include the labeled primers of Mansfield to achieve sensitive detection of amplification products.

Response to Arguments

Applicant's arguments filed April 6, 2009 have been fully considered but they are not persuasive.

Applicant traverses the rejection of claims 1-2, 4-11, 16, 23 and 24 under Backus in view of Bustin and Birch. Applicant argues that "the Backus reference does not teach the combination of a volume exclusion agent and a chemically-modified hot start DNA polymerase for the coamplification of two or more target nucleic acids wherein the nucleic acids are present at the beginning of the reaction in comparable copy numbers". Applicant also notes that Backus indicates that "in light of the teaching of Sambrook, et al., one skilled in the art would not use a volume exclusion agent in standard amplification methods as the hybridization rates are fast enough without its use and the target nucleic acids are not present in rate limiting amounts" (p. 12 of remarks). Applicant concludes that "Backus only allows that the use of a volume exclusion agent in combination with an antibody-inactivated polymerase may be suitable under very limited hybridization conditions, e.g., where there is approximately 100,000 fold difference in levels of target nucleic acids" and points to Examples 1-4. Then Applicant contrasts Backus against their invention and conclude that "Applicants' examples demonstrate that use of this combination leads to amplification of multiple targets instead of differential amplification, even where the copy numbers of the targets are comparable and even where the primers used are added in equimolar concentrations" (p. 12 of remarks).

Regarding Bustin, Applicant argues that Bustin "provides a method for determining how badly a multiplex amplification has gone by including an internal standard as a target. No provision for improving the results of multiplex amplification is provided, and no teaching that

can be combined with the Backus reference suggests the improvement claimed by Applicants" (p. 13 of remarks). Applicant also asserts that "Bustin does not disclose any means for leveling the output of a multiplex PCR" and applicant cites from p. 185 of Bustin as an indication that at the time the review was published in 2000, Applicants improved results were unknown. Applicant concludes by noting the teaching of Birch and that "the Birch et al reference in combination with Backus and/or Bustin does not make up for the lack of this teaching" (p. 14 of remarks).

These arguments have been considered, but are not persuasive. First, regarding Applicant provides a mischaracterization of Backus. While Applicant is correct that Backus exemplifies and teaches coamplification of targets that are not present at comparable copy numbers in the presence of a volume exclusion agent, characterizing that Backus only "allows" the combination of volume exclusion agent and hot start polymerase is only suitable under "very limited hybridization conditions" simply based on the embodiments that Backus chooses to exemplify is incorrect. As Backus states "The following examples are included to illustrate the practice of this invention, and are not meant to be limiting in any way". Backus draws no such conclusion as asserted by Applicant that the antibody-inactivated polymerase is only useful under limited circumstances.

Furthermore, Applicant's fixation on the passage **from Sambrook** cited and discussed by Backus is also not a fair representation of the teaching of the Backus reference. The passage from Sambrook is cited as a direct contrast with the teachings of Backus. Stated another way, Backus is clearly establishing that the problems with the inclusion of volume exclusion agents noted by Sambrook **do not apply to the teachings of Backus** (emphasis added). The same

passage of Backus also states "the advantages of the invention are achieved by including a water-soluble or water-swellable, non-ionic, polymeric volume exclusion agent within the amplification reaction" and that "the presence of this agent effectively allows the user to reduce the amount of primer needed for efficient amplification of the nucleic acids, which reduction then allows manipulation of the procedure to procedure so one nucleic acid is amplified preferentially". Backus also concludes that the volume exclusion agent "allows manipulation of the coamplification of multiple target nucleic acids even further" (col. 4, lines 30-47). The cited passage clearly indicates that Backus chooses to manipulate the amplification reaction to coamplify targets that are present in disparate concentrations and also that the process can be manipulated to achieve other results and types of coamplification. This passage indicates that the technique of including the volume-exclusion agent allows flexibility in amplification design and **does not** in any way indicate that the method is only applicable to targets that are present at 100,000 fold difference in concentration. Instead, Backus leaves room for other embodiments of coamplification of multiple targets. Therefore, Applicant's arguments regarding Backus are wholly unpersuasive.

Next, while Applicants' arguments regarding Bustin and Birch are noted, they are also not persuasive. While Applicants argues that Bustin indicates that multiplex amplification is problematic (p. 13 of remarks, which cites specifically to p. 185 of Bustin), Applicant overlooks that Bustin also clearly suggests in the passage that multiplex amplification is more successful when the levels of mRNA are similar. Therefore, Bustin indicates the coamplification of multiple targets that are present at comparable levels is preferrable in RT-PCR and this teaching is readily combinable with the coamplification of multiple targets in the presence of a volume-

exclusion agent and a modified polymerase, as taught by Backus. This combination of references renders the claims obvious, regardless of the context in which Bustin discloses the concept of coamplification of targets present at comparable copy number. Applicants' arguments regarding Birch are also not persuasive. The rejections are maintained.

Applicant traverses the rejection of claims 12-15 as being obvious over Backus, Bustin and Birch further in view of Reed and Demeke and traverses the rejection of claim 25 as being obvious over Backus, Bustin and Birch and further in view of Ivanov. Applicant argues that "Demeke and Reed, alone or in combination with Backus and/or Bustin, and/or Birch et al do not teach or disclose Applicants' method of multiplex PCR" (p. 15 of remarks).

Regarding the rejection in view of Ivanov, Applicant argues "Ivanov relates to PCR reactions in general and not specifically to multiplex PCR methods, and does not contemplate or disclose the particular improvement of the present invention which relates to a means for obtaining uniform multiplex PCR results" and concludes "a person skilled in the art would thus not take from Ivanov et al any solution for the many problems associated with multiplex PCR" (p. 15 of remarks).

These arguments have been considered, but are not persuasive. Effectively, Applicants' arguments above address the same basis for argument applied over Backus, Bustin and Birch above and indicate that the secondary references do not remedy the deficiencies in the combination, as argued above. These arguments are not persuasive for the same reasons as applied above. The rejections are maintained.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEPHANIE K. MUMMERT whose telephone number is (571)272-8503. The examiner can normally be reached on M-F, 9:00-5:30. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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